Supporting Information for

Direct Tracking of Amyloid and Tau Dynamics in Neuroblastoma Cells using Nanoplasmonic Fiber Tip Probes

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Localized surface plasmon resonance (LSPR)

The nanoplasmonic fiber-tip-probe (nFTP) is a tapered optical fiber that has a ~50 nm tip diameter [1]. A single gold nanorod attached to the tip exhibits a bright red spot under white light excitation (**Supporting Figure S1**). The brightness of the gold nanorod arises from the resonant coupling of light to the collective oscillation of the conductive electrons on gold surface, thus is called localized surface plasmon resonance (LSPR). The scattering cross section is several orders of magnitude larger than fluorescent molecules [2]. Since the resonance spectrum comes from coupling to the surface electrons, it is highly sensitive to small perturbations (refractive index change) on the sensor surface.

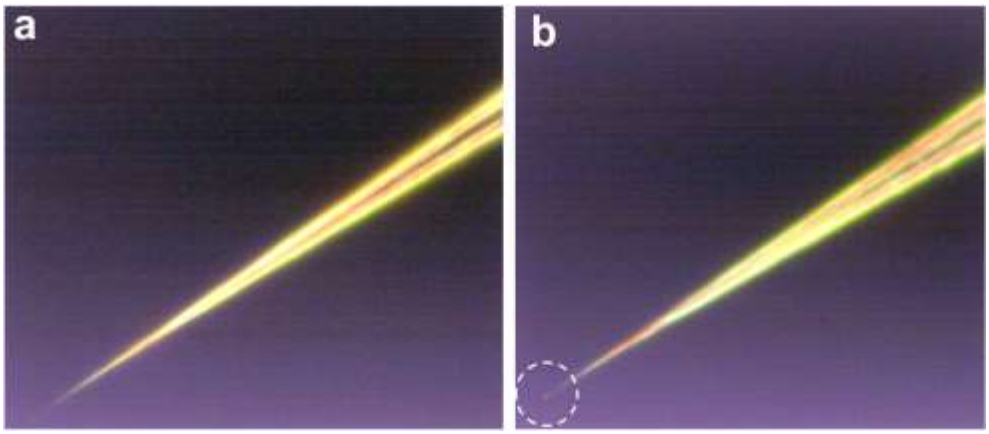

Figure S1. Optical image (20X) of the nFTP without (**a**) and with (**b**) a single gold nanorod on its tip. White light source excites a bright red spot as circled in (**b**), due to strong LSPR.

Surface functionalization and non-specific binding

The gold nanorod surface was functionalized with antibodies using Thiol chemistry as described in **Method**. It was then incubated for 20 minutes in the cell culture medium in which fetal bovine serum (FBS) was replaced by 1% bovine serum albumin (BSA), which blocked the uncovered possible binding sites on the gold surface, thus minimizing the non-specific binding. **Supporting Figure S2** showed the localized surface plasmon resonance (LSPR) shift using nanoplasmonic fiber tip probe (nFTP) to detect 100 nM total-tau, tau-PS262 and Aβ42 proteins in the cell culture medium with and without the blocking step. The nFTPs without blocking showed almost twice of LSPR shifts. The data indicated that non-specific binding was strong in the complex solution, and blocking was an indispensable step.

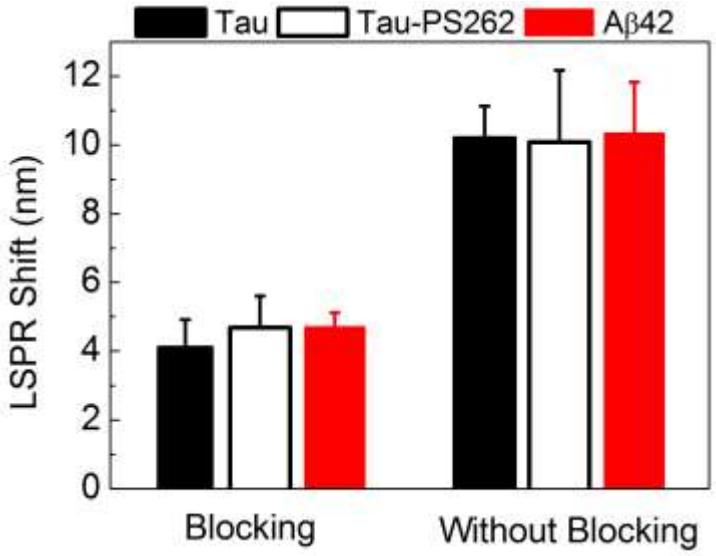

Figure S2. Blocking refers to incubating the sensor in 1% BSA in cell culture medium (without FBS) after conjugating antibodies: anti-tau (ab24634, Abcam), anti-tau-PS262 (sc-32828, Santa Cruz) and anti-Aβ42 (ab10148, Abcam). The nFTP was employed to measure 100 nM of tau, tau-PS262 and Aβ42 proteins in the cell medium. Without blocking, nearly half of the sensor signal came from the non-specific binding.

Verification of antibody attachment on the sensor surface

Prior to the time dynamics measurement on the single cell (or neuron), we performed the blocking step and tested the nanoscale fiber tip probe (nFTP) in the cell culture medium with 100 nM concentration tau-PS262 (Santa Cruz, sc-32828p), as shown in **Supporting Figure S3**. This process verified that antibody was successfully coated on the sensor surface.

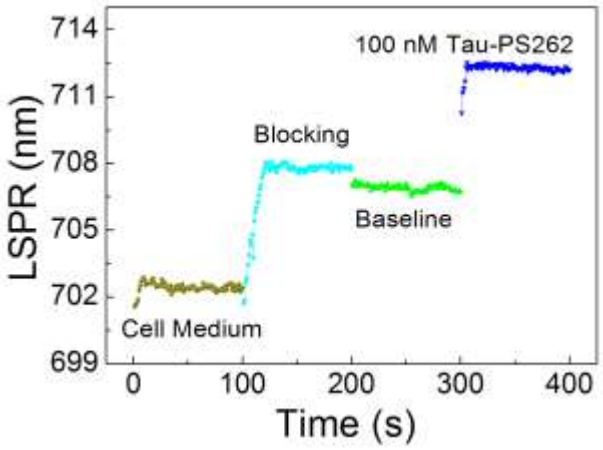

Figure S3. Tau-PS262 antibody was coated on the nFTP. The LSPR signal was first measured in the cell culture medium in the absence of fetal bovine serum (FBS) (brown). Then 1% BSA was mixed into the cell culture medium (without FBS), which was used to block the possible non-specific binding (cyan). Next, the LSPR was measured again in a blank cell culture medium (without FBS) as the baseline (green). Finally, the LSPR was measured in 100 nM tau-PS262 mixed into the cell culture medium (without FBS) (blue).

Sensor regeneration

Regeneration refers to the process of washing off the bound proteins from the capturing antibodies while preserving the capturing antibodies on the sensor surface, thus resets the biosensor for repeated measurements. **Supporting Figure S4** showed repeated detection and reliable regeneration of total-tau proteins. The baseline was the cell culture medium [without fetal bovine serum (FBS)]. Repeated measurement was taken in the 100 nM total-tau pre-mixed to the cell culture medium (without FBS). Regeneration solution was glycine with pH 2.5 tuned by hydrogen chloride acid.

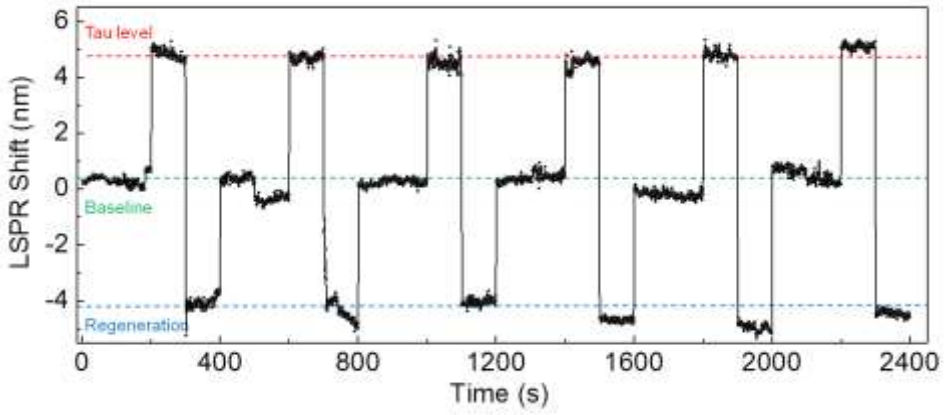

Figure S4. The regeneration solution was glycine with pH = 2.5 (tuned with hydrogen chloride acid). Baseline was the cell culture medium (without FBS). Test solution was 100 nM total-tau proteins mixed into the cell culture medium (without FBS).

Sensitivity and dynamic range of the nanoplasmonic fiber tip probe (nFTP)

The sensitivity and dynamic range of the nanoscale fiber tip probe (nFTP) biosensor can be tested by mixing different concentrations of Aβ42 (ab120301, Abcam) and tau-PS262 proteins (Santa Cruz, sc-32828p) into the cell culture medium. The nFTP biosensor was functionalized with anti-Aβ42 (ab10148, Abcam) and anti-tau-PS262 (sc-32828, Santa Cruz). **Supporting Figure S5** showed the localized surface plasmon resonance (LSPR) shift at different concentrations of Aβ42 (**a**) and tau-PS262 (**b**) mixed into the cell culture medium [without fetal bovine serum (FBS)]. The nFTP biosensor was regenerated between different concentrations.

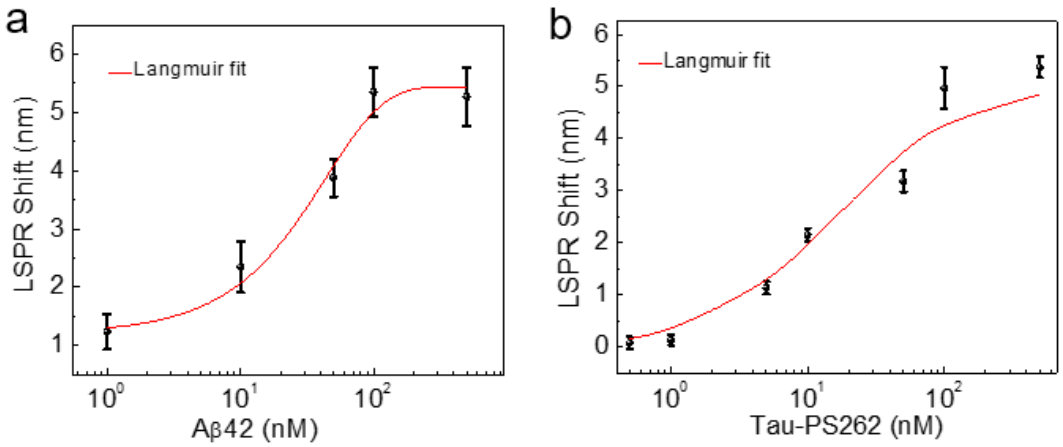

Figure S5. LSPR shift at different concentrations of Aβ42 (**a**) and tau-PS262 (**b**) solutions fitted with the Langmuir equation [3]. The affinity between Aβ42 and its antibody is 44 ± 12 nM. The affinity between tau-PS262 and its antibody is 16 ± 4 nM.

Mechanically revolving dual-nFTP

Supporting Figure S6 showed a mechanically revolving dual-nFTP (nanoscale fiber tip probe) device. A home-built revolving cylinder, 120º rotatable, held three metal fiber chucks (two are shown). Each chuck held one nFTP device. In our experiment, we used two nFTPs functionalized with Aβ42 antibody (ab10148, Abcam) and phospho-specific tau antibody (anti-tau-PS262, sc-32828, Santa Cruz), respectively.

Figure S6. Photograph of a mechanical revolving dual-nFTP device. Each fiber chuck holds 125 μm optical fiber (Thorlabs), and can be rotated by 120º with the revolving cylinder.

K252a inhibitor tested by Western blot

We used Western blot to verify K252a as a tau-PS262 inhibitor. The SH-SY5Y-tau cells were treated with isoflurane for 6 hours (**Supporting Figure S7**), as compared to the 2 hour isoflurane treatment in the nFTP studies. This is because, with Western blot, we were not able to observe significant difference in tau-PS262 level between the control and isoflurane group without increasing the isoflurane treatment time from 2 hours to 6 hours. When 3 μM K252a was used to treat SH-SY5Y-tau cells for 1 hour before the isoflurane treatment for 6 hours, the tau-PS262 level was apparently suppressed, in consistent with the data from the studies by Le Corre et. al. [4].

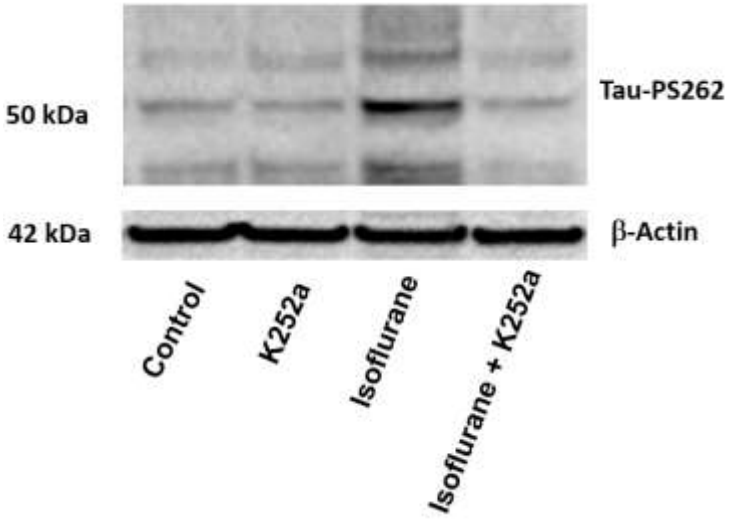

Figure S7. K252a attenuates the isoflurane-induced tau-PS262 protein increase in SH-SY5Y-tau cells. Treatment with 2% isoflurane for 6 hours increases tau-PS262 level as compared to the control condition. The treatment of K252a alone does not induce tau-PS262 level change as compared to the control condition. 3 μM K252a treatment attenuates the 2% isoflurane-induced tau-PS262 level increase as compared to 2% isoflurane alone.

Fabrication of photonic crystal nanosensors

The silicon photonic crystal nanobeam cavity consists of a tapered array of holes (periodicity 330nm), defined along a 600 nm wide ridge waveguide. The device was fabricated on a silicon-on-insulator (SOI) wafer with a 220 nm device layer on a 3mm buried oxide. The cavity region was approximately 15 μm long. The diameters of the gratings are quadratically tapered from 220 nm to 188 nm using 25 periods. 5 additional linearly tapered gratings (from 188 nm to 85 nm) were added to facilitate efficient light coupling from cavity to the waveguide. The device was defined by electron-beam lithography using Hydrogen SilsesQuioxane (HSQ) as the resist, followed by reactive ion etching (C4F8/SF6) of silicon. In order to achieve efficient coupling between a commercial tapered optical fiber and the silicon waveguide, a polymer fiber-waveguide coupler was employed. The fiber-waveguide coupler is a 3 μm by 3 μm cross-section polymer (SU-8) waveguide, defined through a second electron-beam lithography step. In the mode conversion section, the width of the silicon waveguide was linearly tapered from 600 nm to 50 nm over a length of 500 μm, in order to adiabatically couple the light from the SU-8 waveguide to silicon waveguide. The spectrum was obtained by sweeping the tunable laser (Santec) and collecting the transmitted signal from the cavity with a photodetector.

Surface functionalization of photonic crystal nanosensors

First, a 2% solution of 3-aminopropyltrimethoxysilane (APTES) in 95% ethanol was injected into the microfluidic channel for 10 minutes, followed by a removal of residual siloxane by flushing with 95% ethanol. Next, 10 mM of glutaraldehyde (Sigma) and 10 mM of sodium cyanoborohydride (Sigma) pre-mixed solution were injected for 2 hours, followed by a wash with phosphate buffered saline (PBS). This created aldehyde termination on the sensor surface. 1 μg/mL anti-tau-PS262 (sc-32828, Santa Cruz) or anti-Aβ42 (ab10148, Abcam) was injected in to the flow channel and incubated for an hour, followed by washing with PBS for 5 minutes.

SH-SY5Y-tau cell lysis

SH-SY5Y-tau cells were cultured until they reached 90% confluency, and then treated by 2% isoflurane for two hours (**Method**). Cell lysis was performed on different cell cultures every two hours after the isoflurane treatment. To lyse the cells, cells were first washed twice with cold PBS. Then cell lysis buffer (ab152163, Abcam, diluted 10 times) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) was added. For tau-PS262 extraction, additional 1 % (v/v) phosphatase inhibitor cocktail 2 (P5726, Sigma) and phosphatase inhibitor cocktail 3 (P0044, Sigma) solutions were added. The cell lysates were sonicated for 15 seconds, and then spun at 18,000 rcf at 4°C for 10 minutes. The supernatant which contains Aβ42 and tau-PS262 proteins were stocked at -80 °C.

References:

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